THE UPTAKE OF I^{131} BY THE THYROID GLAND OF TURTLES AFTER TREATMENT WITH THIOUREA [1]

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The chemical structure and relationships to goitrogenicity of several hundred compounds have been tested by a number of investigators, for example: the Mackenzies (1943); Astwood, Sullivan, Bissell and Tyslowitz (1943); Astwood (1943); Taurog, Chaikoff and Franklin (1945); Astwood, Bissell and Hughes (1945); McGinty and Bywater (1945) and VanderLaan and Bissell (1946). In general the active substances fall into one of three classes: 1) thiourea and its derivatives, 2) aniline derivatives, including the sulfonamides and other aminobenzene compounds, 3) thiocyanates and organic cyanides. The most active compounds tested possess a thiourea grouping or thioureylene radical —NH·CS·NH—. Replacement of the hydrogens of thiourea by methyl groups increases its activity, a fact which suggests the importance of the thio rather than the mercapto grouping for the activity of this class of substances. Extensive reviews concerning antithyroid agents have been published by Charipper and Gordon (1947), Astwood (1949) and Comsa (1953).

Vertebrates of every class have been tested for their reaction to antithyroid drugs. Such studies, however, for the most part, have dealt with mammals. Gordon, Goldsmith and Charipper (1943) made the first report of the use of inhibitors on the thyroid gland of cold-blooded animals. Since then a number of investigators have studied the effects of goitrogenic substances on poikilotherms. Lynn and Wachowski (1951) have published a comprehensive review of the literature dealing with the thyroid gland and its functions in cold-blooded vertebrates.

Little work has been done concerning the function of the thyroid in reptiles. Ratzersdorfer, Gordon and Charipper (1949), Adams and Craig (1951) and Fisher (1953) have reported the effects of antithyroid compounds on the lizards. Naccarati (1922) described the normal histology and gross anatomy of the thyroid gland of the turtle *Emys europa*, while Evans and Hegre (1940) studied seasonal changes and the effects of pituitary extract on the thyroid of *Chrysemys picta belli*. Greenberg (1948) was the first to investigate the effects of thiourea on the histology of the thyroid gland of the turtle. In her work immature specimens of *Pseudemys elegans* were used. Since then, Adams and Craig (1950) and Paynter (1953) have studied the thyroidal response to goitrogens in *Chrysemys picta picta*. Pastore

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(1950) investigated the effects of thyroid-stimulating and thyroid-inhibiting drugs upon the histology of the thyroid of *Clemmys insculpta* and *Graptemys geographica*. Dimond (1954) studied the reactions of developing snapping turtles, *Chelydra serpentina serpentina*, to thiourea.

Investigators who have worked on the thyroid gland of turtles are unanimous in pointing out, as an explanation for the observed irregular reactions to goitrogenic agents, what appears to be an inherent variability. This variability far exceeds that found in warm-blooded vertebrates. Uhlenhuth, Schenthal, Thompson, Mech and Algire (1945) working with the newt, *Triturus torosus,* claimed that such a high degree of variation does not seem explicable on the basis of the known physiological roles of the urodele thyroid and must be attributed to what might be called a general instability of the endocrines in cold-blooded vertebrates.

Some of the methods used to acquire a more thorough knowledge of the gland are: 1) gravimetric methods, based on changes in thyroid weight; 2) chemical methods, a quantitative as well as a qualitative study of iodine in the gland; 3) the use of radioisotope technique (radioactive iodine in the thyroid increases progressively with increasing dosage of TSH); 4) histological methods, which study mainly: a) epithelial height, b) staining reactions of colloid and cells, c) position and shape of nuclei and nuclear volume; 5) microhistometric methods. Uotila and Kannas (1952) have devised a linear measurement method, which permits quantitative determinations of the principal components of the thyroid tissue. The method appears to have the advantage of objectivity, simplicity and economy of time. This method was compared with the planimetric method and cell height measurement by Tala (1952) and found to give an accurate picture of the histological activity of the thyroid gland.

The use of radioactive iodine in experimental studies on the thyroid gland has added considerably to present-day understanding of the histophysiology of this organ. The early studies of Hertz, Roberts, Means and Evans (1940), Hertz and Roberts (1941), and Hamilton and Soley (1940), in which radioactive iodine was used for the first time in thyroid investigation, ushered in a technique which enabled more precise interpretation of the relationship between iodine metabolism, the thyroid and the hypophysis.

Tracer studies, using radioiodine after treatment with thyroid-inhibiting and thyroid-stimulating substances, are perhaps the most widely applied tools in thyroid investigation today. These recent developments are being used extensively in studying the thyroid function in mammals and are now being employed to some extent on cold-blooded vertebrates. However, as stated above, very few experiments have been performed on the reptiles. This is a particularly significant gap in our knowledge, in view of the fact that reptiles are considered as the stem of the birds and mammals in the vertebrate scale, and as the only cold-blooded amniote. One of the main reasons for this neglect has been the difficulty in performing thyroidectomy in these animals. The recent development of an effective "chemical thyroidectomy" opens new opportunities for research in this field. Already, some attempts have been made at correlating cell height, dry weight of the thyroid and the histological picture with the uptake of radioiodine in cold-blooded vertebrates such as salamanders (*Desmognathus fuscus* (Rafinesque); Fisher, 1953) and in the turtle *Chrysemys picta picta* by Paynter (1953). The results obtained in these experiments point again to the greatest difficulty encountered in the study of thyroid function at this level, namely, the astonishing variability. The present work is an attempt at investigating some of the factors which may influence this great variability in the function of the thyroid of the turtle *Chrysemys picta*. To this effect a study was made of the possible correlations between radioiodine uptake and colloid level, percentage epithelium, cell height and dry weight of the thyroid, in normal and in treated animals; an endeavor was also made to determine the thyroid/serum ratio, when organic binding is blocked and when binding is permitted.

Materials and Methods

Turtles with carapace length between five and seven inches were purchased from The Lemberger Company, Oshkosh, Wisconsin and the J. R. Schettle Frog Farm, Stillwater, Minnesota.

The experimental and control animals, totaling one hundred sixteen, were kept in large metal tanks which were arranged so that the animals had free access to running water or dry perches. The temperature of the room in which the tanks were located was kept as close to 75° F. as possible. All animals used in this series of experiments were denied food during the term of treatment since the amount of iodine in the food could not have been controlled.

The experimental animals were given subcutaneous injections of thiourea three times a week. The dosage, found most effective by Paynter (1953) for these experiments, was 0.25 cc./100 gr. of body weight of a 0.1% solution. After four, six and eight weeks of treatment the animals were injected with a tracer dose of three microcuries of carrier-free I^{131}, in 0.5 cc. of distilled water. The untreated control animals received the same dose of I^{131}. Three hours after administration of the radioactive iodine the animals were sacrificed. The plastron of each turtle was removed, blood was taken from one of the large vessels leaving the heart and allowed to clot. Immediately after exsanguination, the thyroid gland was removed and cut in half. The right half was cooled by placing it on dry ice. The left half, in 27 of the experimentals and 20 of the control animals, was immersed in isopentane, cooled by liquid nitrogen. Afterward, the glands, placed in individual tubes, on the surface of degassed paraffin, were transferred to the drying chamber of a freezing-drying apparatus (Altmann-Gersh technique; Gersh, 1932). Dehydration was carried out at − 40° C. and continued for a minimum of 72 hours. The system was then gradually brought to the melting point of paraffin by immersing the drying chamber in a beaker of water which was kept at constant temperature by placing it on a thermostatically controlled hot plate. The vacuum was broken only after infiltration was completed.

As for the remainder, 42 experimental and 27 control animals, the left half of the gland was placed on a glass slide and dried at 37° C. to determine dry weight. These glands were recovered by soaking in 0.025% solution of trisodium phosphate for 24 hours and in 10% formalin for three hours.

The right half of the gland, for each animal, was placed into glass homogenizers containing 6 ml. of ice-cold distilled water and one mg. of NaI as carrier, and homogenized immediately. The homogenate was deproteinized according to Somogyi (1945). To 0.80 cc. of homogenate, 0.10 cc. each of $Ba(OH)_2$ and $ZnSO_4$ were added, followed by vigorous shaking, and centrifuging. The supernate was removed with a pipette, three drops were placed on each of three glass sides. The same was done with the precipitate and a sample of the homogenate before deproteinization. These were dried at 37° C.

The serum was deproteinized by diluting a 0.05-ml. sample to 0.08 ml. with water containing 0.10% NaI and adding to it 0.10 ml. each of $Ba(OH)_2$ and $ZnSO_4$ as above. To determine the I^{131} content of the serum an aliquot of diluted serum (0.05 ml. diluted to 1.00 ml. with water containing 0.10% NaI) was dried on a glass slide.

Precipitable and non-precipitable I^{131}, as well as total I^{131} in both gland and serum, were measured with a Geiger-Mueller tube (window thickness 1.7 mg./cm.$^{-2}$) placed at a distance of 4 mm. from the object. The counts were also determined for the glands of both series, frozen-dried and oven-dried. Three determinations of one minute duration each were taken and averaged. The counts were brought up to the value on the day of injection by the use of decay factors which were based on the eight day half-life of I^{131}.

The glands were sectioned at 5 micra and those of the frozen-dried series were fixed by floating on 10% formalin and amphibian Ringer's solution and stained with Gomori's chrome alum hematoxylin and phloxin. Three sections were selected at 25%, 50% and 75% of each gland. Film strips of these sections were made and projected on white paper which served as a screen, using a magnification of × 100. Two lines, intersecting at obtuse angles in the form of an ×, were drawn in advance on the plane to which the image was to be projected. The image of the stained specimen was positioned so that its center fell approximately on the junction of the intersected lines. The outline of the follicles and colloid along the full length of the two lines was drawn. The segment of the lines covered by the entire figure was then measured in millimeters. Similarly, millimetric lengths of the segments covered by epithelium and colloid were determined. The total of the epithelium segments, divided by the whole length of the lines, gave the percentage of epithelium. The percentage of colloid was calculated in the same way. This linear measurement method of determining the principal components of the thyroid gland was devised by Uotila and Kannas (1952) and further tested by Tala (1952).

Observations

It is fairly well established from animal experimentation that antithyroid compounds of the thioureylene type owe their activity to their property of preventing the organic binding of iodine, or, what amounts to the same thing, the inhibition of the oxidation of iodide to iodine. Under the influence of these agents, iodide is still able to concentrate in the thyroid gland, but it remains in a reduced, ionic state. As a result of treatment with thiourea it should then be expected that radioiodine uptake would be higher in the experimental animals than in the corresponding control groups. This fact was well brought out in the course of the study; in all cases the counts were much higher in the treated animals than they were in the untreated. Analysis of the results based solely on length of treatment were much too variable to draw significant conclusions. Re-interpretation of these results on the basis of seasonal cycles gave a more valid picture.

A total of 72 animals were treated with thiourea; 52 of them received 18 subcutaneous injections over a period of six weeks and 20 received 12 injections over a period of four weeks. Forty-seven animals were used as controls and received three microcuries of radioiodine at the same time as the treated animals, three hours before they were killed. One set of controls was examined each time an experimental group was run.

An analysis of the results from two different points of view, 1) length of treatment and 2) seasonal factor, follows.

Length of treatment

From this point of view, the outstanding feature throughout the entire study is the great variability observed in control groups as well as in experimental animals. Taking the various correlations specifically the following can be reported.

Six-week series. The percentage epithelium was lower and the range of variation in percentage epithelium was considerably higher in experimental animals. The radioiodine uptake per unit epithelium and the range of variation in uptake were both higher in the treated than in the control group. The coefficient of correlation between the per cent epithelium and radioiodine uptake was − 0.38 for the experimental group and − 0.31 in the control animals. It becomes evident from the above figures that the correlations between per cent epithelium and uptake were practically nil in both treated and untreated groups.

The percentage colloid, radioiodine uptake per unit per cent colloid, and the range of variation were considerably higher in the treated animals than in the control group. The coefficient of correlation for the colloid and uptake was − 0.19 in the treated animals, while it was − 0.69 in the control group. In terms of colloid uptake the correlation was slightly improved in the treated animals but remained insignificant in the control animals.

Four-week series. There was no significant difference between the epithelium percentage and colloid level of experimental and control animals. The radioiodine uptake was much lower than in the six-week group and the range of variation was greatly reduced. The coefficients of correlation were as follows: per cent epithelium and uptake, control − 0.20, experimental, − 0.06; unit colloid and uptake, control − 0.10, and experimental − 0.27. The correlations in the four-week series were considerably improved but even if the range of variation was reduced it was still too high to permit significant correlations.

From the point of view of length of treatment it was impossible to establish significant correlations, due to the high range of variation. The correlations were poorest in the six-week series, but became somewhat improved in the four-week series. This was particularly true in regard to colloid level and iodine uptake.

Seasonal factor

During the course of this study the influence of a seasonal factor has been observed. Since the animals had been obtained from the supplier at three different times of the year, the three series therefore varied as to the time of the year during which the experimental work was done. When the results were analyzed in terms of seasonal cyclic activity, the correlation between epithelium or colloid and uptake was greatly improved and the variability was decidedly reduced, especially in the fall series. The first series was carried out during June and the beginning of July. The second series was carried out during November and the last series during the month of February and the beginning of March. As a result of such spacing, the

TABLE I

Radioiodine uptake per milligram dry weight. Difference in per cent of controls

Series	Mean count per milligram		% of control uptake
	Experimental	Control	
Winter	592.29	527.01	112.38%
Summer	985.34	534.85	184.22%
Fall	484.16	378.55	127.89%

possibility of a seasonal factor controlling the activity of the thyroid gland in the turtle was brought out, and this factor appeared to exercise its influence even in the treated animals.

Uptake per milligram dry weight. In the determination of radioiodine uptake, it was observed that 1) the counts per minute per milligram dry weight were higher in the treated animals than in the corresponding control animals, and 2) the counts for both experimental and control animals were highest during the summer and lowest in the fall series. This last point is a strong indication of the influence of a seasonal factor and is in agreement with Eggert (1935) who reported highest thyroid activity in June, in the case of hibernating lizards, and lowest activity during December and January. In this case the winter group, having been killed at the end of February and the beginning of March, would correspond to the resumption of the secretory activity. A summary of the results in per cent of control is given in Table I.

Thyroid/serum ratio. Measurements of the thyroid/serum ratios were made when organic binding was blocked with thiourea and when binding was permitted. Due to the high degree of individual variation, the time required for the maximum uptake, as determined by Paynter (1953), was found to vary considerably with

TABLE II

Per cent epithelium. Experiments in the order of their ranges of variation

Series	Seasons	No. of cases	Mean % epithelium	Maximum–minimum	Range	
					In points	In per cent of mean
Experimental	Winter 6 weeks	12	15.18	18.33–11.44	6.89	45.38%
	Summer 6 weeks	11	16.18	20.90–10.27	10.63	65.69%
	Fall 4 weeks	18	16.22	18.18–14.26	3.92	24.16%
Control	Winter	9	17.01	20.09–12.86	7.23	42.50%
	Fall	13	16.77	19.84–12.70	7.14	42.57%

each animal. Consequently a significant ratio could not be established. The effects of the seasonal cycle, however, could still be observed. In taking the counts per minute for the homogenate and comparing it with the counts for the serum the following results were obtained:

1) The summer group: in 92% of the experimental and 40% of the control animals, the thyroid homogenate had a higher count than the serum.

2) The fall group: in 43% of the experimental and 20% of the control animals, the thyroid homogenate had a higher count than the serum.

3) The winter group: in 36% of the experimental and 16% of the control animals, the thyroid homogenate had a higher count than the serum.

The results bring out very clearly the influence of a seasonal cyclic activity. The values obtained for the summer groups are almost three times as high as those for the winter groups and twice as high as the values obtained for the fall series. These differences appear to be directly correlated to the phase of activity of the thyroid gland in hibernating reptiles.

TABLE III

Colloid level. Difference between ranges of controls and experimentals

Season	Series	Colloid level Maximum minimum	Range	Difference between experimental and control ranges	
				In points	In % of control range
Winter	Control	75.50 63.12	12.38		
	Experimental	78.93-54.86	24.07	+11.69	+94%
Fall	Control	80.87-67.59	13.30		
	Experimental	78.74-60.67	18.07	+ 4.77	+35%

Per cent epithelium and iodine uptake. Two six-week series were carried out, one during late February and early March and the other during June and July, and one four-week series during November. The range of variation was too high to establish significant correlations between the per cent epithelium and radioiodine uptake, but the influence of the seasonal factor was nevertheless observed. The per cent epithelium in the treated animals was higher in the summer, with no significant difference in percentage for the fall group, but decidedly lower for the winter. In the control series, comparisons between percentage epithelium or colloid level are available only between the fall and winter groups. The summer control animals were not used for measurements. The range of variation in the three series gave a definite indication of a seasonal cyclic influence. Table II lists the per cent epithelium with the range of variation for the three seasons during which the work was carried out.

The uptake of radioiodine per unit per cent epithelium was considerably higher during the summer and lowest during the fall. Here again the results seem to indicate that the state of activity of the thyroid gland in the painted turtle corresponds

TABLE IV

Radioiodine uptake per unit colloid. Difference between ranges of controls and experimentals

Season	Series	Radioactivity Maximum minimum	Range	Difference between experimental and control ranges	
				In points	In % of control range
Winter	Control	122.74–10.88	111.86		
	Experimental	238.24– 6.58	231.86	+119.82	+107%
Fall	Control	197.51– 4.59	192.92		
	Experimental	129.76– 6.58	123.18	– 69.74	– 36%

rather closely to the seasonal cycle as described above and in agreement with the work of Eggert (1935).

The counts per unit epithelium in the treated animals were 383.63 for the summer, 335.79 for the winter and only 157.13 for the fall groups. Comparing the counts for the untreated animals the same general cyclic activity could be observed. The correlations, even though somewhat improved, still remained insignificant because of the range of variation.

Colloid level. The influence of the seasonal factor became more evident in the studies on correlation between colloid level and radioiodine uptake. The cyclic pattern was decidedly in accordance with the various phases of activity described for the hibernating reptiles. The mean uptake per unit colloid level was 79.91 for the summer, 65.31 for the winter, and 33.86 for the fall group in the treated animals.

The best evidence for this factor was brought out in the closer correlation be-

TABLE V

Colloid level experiments in the order of their ranges of variation

Series	Seasons	No. of cases	Mean % colloid	Maximum minimum	Range	
					In points	In per cent of mean
Experimental	Winter 6 weeks	12	69.46	78.93–54.86	24.07	34.65%
	Summer 6 weeks	11	75.95	87.01–60.54	26.47	34.86%
	Fall 4 weeks	18	72.76	78.74–60.67	18.07	24.83%
Control	Winter	9	69.24	75.50–63.12	12.38	17.87%
	Fall	13	71.81	80.87–67.59	13.30	18.52%

tween the colloid level and the uptake of radioiodine in the fall group. The range of variation is at its lowest in both colloid level and range of uptake. Tables III and IV give the difference between the ranges of variation for colloid level and uptake in per cent of control range. It is to be observed that for the fall group the variation in colloid level for the experimental group was 35% above the control, as compared with 94% for the winter series. The range of variation of iodine uptake in experimental animals for the fall series was 36% below the controls and 107% above in the winter series.

From the above observations it was possible to conclude that in terms of length of treatment, the range of variation in uptake of radioiodine, either per unit of epithelium or colloid level, was much too high to establish significant correlations. When, however, the results were analyzed from the point of view of seasonal cyclic activity it became apparent that the gland was under the influence of a seasonal factor which exercised its control in treated as well as in untreated animals. More complete data on colloid level and uptake and variation are given in Tables V and VI.

Table VI

Radioactivity/unit % colloid. Experiments in order of time of treatment

Series	Seasons	No. of cases	Mean radio-activity per unit % colloid	Maximum–minimum	Range	
					In points	In per cent of mean
Experimental	Winter 6 weeks	12	65.31	238.24– 6.58	231.68	354.74%
	Summer 6 weeks	11	79.91	141.83–18.69	123.14	154.09%
	Fall 4 weeks	18	33.86	129.76– 6.58	123.18	363.79%
Control	Winter	9	57.99	122.74–10.88	111.86	192.89%
	Fall	13	40.61	197.51– 4.59	192.92	475.05%

Discussion

Antithyroid compounds may inhibit the normal function of the thyroid gland by acting directly on the thyroid itself at any one of the stages of hormone production: 1) the collection of iodide from the circulation; 2) the synthesis of thyroid hormone; 3) the release of hormone to the tissues.

Thiocyanate ions exert a unique effect upon the thyroid gland shared by no other substance yet known (Astwood, 1949). Animals treated with this substance have been shown to be unable to collect iodide from the circulation. Other substances, like thiourea and thiouracil, are believed to block an enzyme system, thereby preventing organic synthesis of the hormone, but having no effect on the "iodide trap."

Astwood and Bissell (1944) found that in rats under the continuous influence of thiouracil the iodine content of the thyroid rapidly falls to low levels and that the thyroid gland simultaneously enlarges. Astwood (1944–45) showed that animals thus depleted of iodine are still able to concentrate rapidly considerable quantities of iodine when injected with potassium iodide.

D'Angelo, Paschkis, Cantarow, Siegel and Riviero-Fontan (1951) have observed that despite uniformly decreased radioiodine uptake with chronic propylthiouracil treatment the total radioactivity in the thyroid eventually exceeds normal when sufficient hyperplasia has occurred to offset the limited uptake. The augmented radioiodine collections which result where the drug is withdrawn, however, are greater than would be expected from hyperplasia alone, and must result in part from increased avidity of thyroid tissue for the radioactive iodine. The augmented avidity for iodine upon withdrawal of the drug is demonstrable after periods of treatment too short to have caused hyperplasia, although it increases progressively with longer periods of treatment and consequently with thyroid hyperplasia before the goitrogen is withdrawn.

The results obtained in this study are in agreement with the above observations. The uptake of radioiodine was considerably higher in the animals in which binding was blocked as compared with those in which binding was permitted. This rapid uptake of iodine by the thyroid gland, reaching a maximum and followed by a discharge of the trapped iodine, varies with the type of animals used. In mammals, for example, the time required for maximum concentration may be as little as ten to fifteen minutes as reported by Hertz, Roberts, Means and Evans (1940) and Chaikoff and Taurog (1949).

In cold-blooded animals, however, great variations are observed in the reactions of the thyroid, and because of this great range of variability the maximum uptake in the turtle would seem to be controlled by a factor other than time alone.

In accordance with Adams and Craig (1950), Paynter (1953) and Dimond (1954) the results obtained in this investigation show that the reactions to thiourea in the turtles are not as profound as those reported in warm-blooded animals, and especially in mammals. The percentage epithelium was slightly lower in treated animals than in control animals, while the colloid was higher in the experimental than in the control animals. This could be explained by the fact that the experimental animals were injected with a tracer dose of I^{131} 24 hours after the last treatment with thiourea. This delay would allow the pituitary-thyroid axis to be restored to normal conditions, and consequently after a period of increased activity, the thyroid had stored a considerable amount of colloid.

Seasonal factor. A discussion of the seasonal physiology of any vertebrate immediately arouses an inquiry concerning the behavior of its endocrine glands, especially that of the thyroid and the pituitary because of the reactions to temperature by the former and the close association and control of the thyroid function by the latter.

In general, throughout the vertebrate classes, it may be said that if a species is inactive (hibernating) during the winter months, the thyroid is inactive at this time and will not become active until the animal resumes activity. Warm-blooded animals for example, which are active all winter, have thyroids reported to be more active during the cold season, in order to maintain their normal body temperature and BMR. The same animals have lower rate of thyroid activity during the summer, the temperature of the environment tending to prevent the loss of body temperature, thereby lowering the energy requirement for the maintenance of a normal BMR.

It can generally be said that the reverse is true in cold-blooded animals. However, the seasonal conditions of thyroids in cold-blooded vertebrate hibernators are less well known. Morgan and Fales (1942) reported that there are comparatively few observations on the full seasonal cycles of the thyroid of amphibians and some of these are conflicting. Several observers are in agreement concerning the seasonal condition of the thyroids in various species of frogs and toads, all of which are hibernators. In the main they report a winter phase of moderate activity and a summer phase of greatly lowered activity. Burger (1946), in his observations on seasonal conditions of the thyroid of the male of three species of urodeles, reports variations between animals and between individual follicles of the same gland. The three species, however, all showed a similar broad cyclic pattern in that activity was highest in the spring, lowest during the summer and moderate in the fall. Similar results have been reported by Miller and Robbins (1955) for the urodele amphibian *Taricha torosa* (*Triturus torosus*).

Although few in number, some studies of the seasonal cycle of the thyroid of reptiles have been made on hibernating and non-hibernating species. Eggert (1935) studied three forms of European Lacertas and reported hibernation beginning from the end of September reaching a peak in December and January when the gland is at its most reduced activity. Young animals resume their secretory activity in February and attain their highest activity in June. Seasonal variations in the thyroid of lizards have also been noted by Ratzersdorfer, Gordon and Charipper (1949) in *Anolis carolinensis*. In non-hibernating *Xantusis vigilis*, Miller (1955) has observed that the cycle is closely correlated with the various phases of the life history of the animal. He reports the lowest thyroid activity for the fall and an increase in activity during the winter. This increase in activity during the winter may be related to the fact that the animals are active and feeding during the coldest months of the year. The influence of the seasonal factor has also been observed in the cyclic activity of the thyroid in turtles. Evans and Hegre (1940), working with *Chrysemys*, obtained results which resembled those of typical hibernating forms even when the animals were fed at regular intervals and kept at room temperature (70° F.) throughout the fall and winter. This they claim would indicate that the thyroid gland of the turtle was under the control of a genetic factor. This factor exercises its influence independently of the temperature of the environment.

During the course of this study the influence of a seasonal factor has been observed which is in accordance with the results obtained by Uhlenhuth, Schenthal, Thompson and Zwilling (1945); Uhlenhuth, Schenthal, Thompson, Mech and Algire (1945); Evans and Hegre (1940) and Greenberg (1948). The experimental animals had been treated with a 0.1% solution of thiourea, a concentration claimed to be most effective by Paynter (1953). It would seem, however, that since turtles and reptiles in general appear to be more refractory to thiourea, a higher dose would produce more marked results. Despite the weak responses and the great range of individual variation, the results were more significant when interpreted in terms of seasonal activity.

The uptake of radioiodine by the thyroid gland was always considerably higher for the summer groups, treated and untreated, whether it was considered from the point of view of uptake per milligram dry weight; uptake per unit epithelium, unit colloid or thyroid/serum ratio. The uptake for the fall and winter series was much lower than that for the summer series. This is in agreement with Eggert (1935) who reports highest thyroid activity in June, in the case of hibernating lizards. The November series then would be nearing the most reduced thyroid activity which in lizards occurs during December and January. The winter group, killed at the end of February and the beginning of March, would then be expected to show resumption of secretory activity. The thyroid gland of the untreated control animals was observed to have a lower uptake of radioiodine per unit; however, the results were shown to follow the same general pattern as the treated animals. These results gave further evidence in favor of the seasonal cyclic activity.

The best correlations were found to occur in the fall series. The epithelium percentages of experimental and control animals were more closely related than in the other series; the range of variation in per cent epithelium was lower in the experimental animals; the correlation between epithelium and radioiodine uptake was definitely improved. The seasonal influence, however, was best demonstrated by the better degree of correlation between the colloid level and the radioiodine uptake. It was observed that the colloid level was lower in untreated controls than in treated thyroids. This may be due to the avidity of the treated thyroids for the iodine, since the injections of I^{131} were given 24 hours after the last treatment with thiourea. Considering the fact that the colloid is known to decrease during the active phase of the thyroid gland, it is not surprising that a lower colloid level was observed in the thyroids of controls for the February–March series than in the November series, which was during the phase of decreasing activity and colloid storage.

Since the turtles were kept in a room where a fairly constant temperature was maintained throughout the experiments, it would seem that the seasonal factor which regulates the cyclic activity of the thyroid in turtles is independent of the environmental temperature and probably is genetic in nature.

Summary

1. The painted turtle, *Chrysemys picta* (Schneider), was treated with a 0.1 per cent solution of thiourea by means of subcutaneous injections, then injected with radioiodine in order to determine the correlation of percentage epithelium and colloid level with radioiodine uptake. The data obtained in this study were analyzed statistically in terms of length of treatment and the time of the year during which the work was carried out.

2. From the point of view of length of treatment, the correlations between the uptake of I^{131} and colloid level or per cent epithelium were very poor, due to the high degree of variability.

3. When these results were analyzed from the point of view of seasonal cyclic activity, the correlations were decidedly improved.

4. The best correlations were obtained in the fall group, where the percentage epithelium of experimental and control animals was more closely related; the range of variation was decidedly lower, and the correlation between colloid level and uptake of radioiodine much better than in the other series.

5. Further evidence in favor of the seasonal cyclic activity was found in the uptake per milligram dry weight, and in the thyroid/serum ratio.

6. This seasonal factor appears to be genetic in nature since it exerts its control independently of the environmental temperature and the effects of the drug.

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